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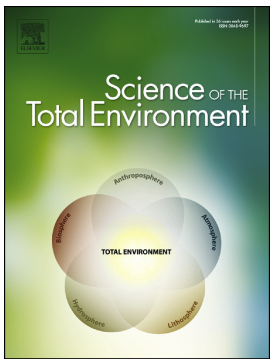

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Chronic nutrient inputs affect stream macroinvertebrate communities more than acute inputs: an experiment manipulating phosphorus, nitrogen and sediment

Running title: Chronic vs. acute nutrient effects

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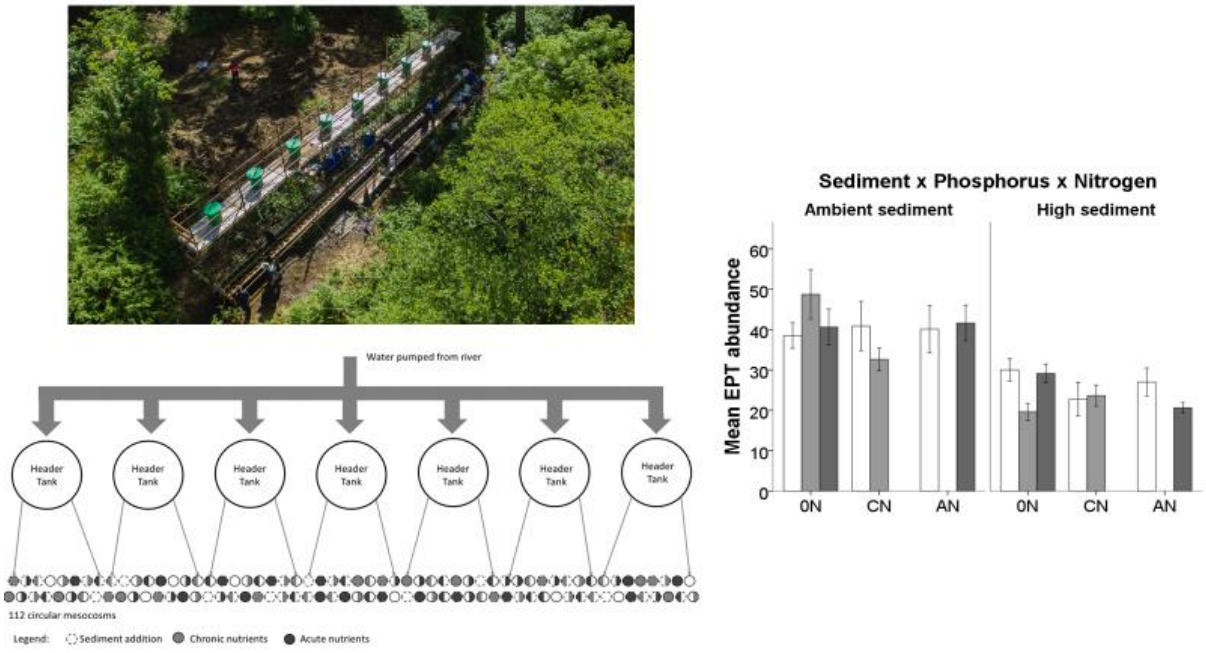
Graphical Abstract



Highlights

- Addressed effects of chronic vs. acute stressors which are poorly understood
- Sediment, phosphorus and nitrogen manipulated
- Chronic nutrients and sediment were added for 18 days
- Two three-hour pulses of acute nutrient (concentrations doubled)
- Responses were generally as a result of chronic exposure and sediment was the most pervasive stressor

Abstract

Freshwaters worldwide are affected by multiple stressors. Timing of inputs and pathways of delivery can influence the impact stressors have on freshwater communities. In particular, effects of point versus diffuse nutrient inputs on stream macroinvertebrates are poorly understood. Point-source inputs tend to pose a chronic problem, whereas diffuse inputs tend to be acute with short concentration spikes. We manipulated three key agricultural stressors, phosphorus (ambient, chronic, acute), nitrogen (ambient, chronic, acute) and fine sediment (ambient, high), in 112 stream mesocosms (26 days colonisation, 18 days of manipulations) and determined the individual and combined effects of these stressors on stream macroinvertebrate communities (benthos and drift). Chronic nutrient treatments continuously received high concentrations of P and/or N. Acute channels received the same continuous enrichment, but concentrations were doubled during two 3-hour periods (day 6, day 13) to simulate acute nutrient inputs during rainstorms. Sediment was the most pervasive stressor in the benthos, reducing total macroinvertebrate abundance and richness, EPT (mayflies, stoneflies, caddisflies) abundance and richness. By contrast, N or P enrichment did not affect any of the six studied community-level metrics. In the drift assemblage, enrichment effects became more prevalent the longer the experiment went on. Sediment was the dominant driver of drift responses at the beginning of the experiment. After the first acute nutrient pulse, sediment remained the most influential stressor but its effects started to fade. After the second pulse, N became the dominant stressor. In general, impacts of either N or P on the drift were due to chronic exposure, with acute nutrient pulses having no additional effects. Overall, our findings imply that cost-effective management should focus on mitigating sediment inputs first and tackle chronic nutrient inputs second. Freshwater managers should

also take into account the length of exposure to high nutrient concentrations, rather than merely the concentrations themselves.

1. Introduction

Freshwaters are amongst the world's most endangered ecosystems (Sala et al., 2000) and river degradation and the loss of biodiversity are of major concern worldwide (Strayer & Dudgeon, 2010; Vörösmarty et al., 2010), with activities such as forestry, urbanisation and agriculture detrimentally affecting the integrity of aquatic ecosystems (Strayer & Dudgeon, 2010). Most river catchments are affected to some degree by agricultural activities (Allan, 2004), which leads to impacts from nutrient enrichment, principally nitrogen (N) and phosphorus (P), and increased fine sediment deposition (Allan, 2004; Matthaei, Piggott, & Townsend, 2010). The effects of stressors acting on freshwater ecosystems has received much attention in recent years (Beermann et al., 2018; Graeber et al., 2017; Lemm & Feld, 2017; Ormerod, Dobson, Hildrew, & Townsend, 2010). The emerging consensus is that there are a multitude of influential stressors, which often interact in complex and unexpected ways (Jackson, Loewen, Vinebrooke, & Chimimba, 2016; Nõges et al., 2016). Consequently, there is a growing recognition that an understanding of how multiple stressors interact and the implications these interactions have for ecological communities are required for effective resource management.

Deposited fine sediment has been shown to affect stream ecosystems by infilling of open gravel substrata (Soulsby, Youngson, Moir, & Malcolm, 2001), leading to declines in habitat quality (Wood & Armitage, 1997), which in turn causes changes in benthic community composition and increases the propensity of stream macroinvertebrates to drift (Larsen & Ormerod, 2010; Rosenberg & Wiens, 1978). Fine sediment often has the strongest detrimental effects on pollution-sensitive groups including Ephemeroptera, Plecoptera and Trichoptera (EPT) (Jones et al., 2012; Larsen, Vaughan, & Ormerod, 2009; Matthaei, Weller,

Kelly, & Townsend, 2006; Rabení, Doisy, & Zweig, 2005; Townsend, Uhlmann, & Matthaei, 2008). Richness and abundances of these groups tend to decline in response to sediment addition (Jones et al., 2012), while in some cases positive effects have been found for pollution-tolerant groups such as Chironomidae (Kreutzweiser, Capell & Good, 2005).

The two main origins of pollution in freshwaters are point sources (e.g. waste water effluent) and diffuse sources (e.g. organic and inorganic nutrients in runoff). Diffuse pollution is usually associated with large, short-lived spikes (typically 3-6 hours) in nutrient and sediment concentrations during storm events (Bowes, Smith, Jarvie, & Neal, 2008; Jordan, Arnscheidt, McGrogan, & McCormick, 2007; Jordan, Melland, Mellander, Shortle, & Wall, 2012). This type of pollution is referred to here as 'acute'. By contrast, point sources pose a more chronic threat, having the greatest effect during the summer months when water levels are generally low (Jarvie, Neal, & Withers, 2006; Withers, Jordan, May, Jarvie, & Deal, 2014), and are referred to here as 'chronic'.

As observed elsewhere in Europe (OECD, 2012), unsatisfactory water quality in Ireland has been attributed to diffuse agricultural pollution and point discharges of waste water (Fanning et al., 2017). Acute pollution events, principally derived from agriculture, often comprise the majority of annual pollutant loads in rural catchments and are typically associated with storm events (Smith, Jordan, & Annett, 2005). However, chronic point-sources are often sufficient to maintain rivers in an eutrophic state between storm events (Jordan et al., 2007). It has also been suggested that chronic nutrient inputs may pose a greater risk to river water quality than acute storm inputs due to the ecological risks associated with symptoms of eutrophication, such as excessive algal growth in times of ecological sensitivity such as summer low-flows, when biological activity is high (Jarvie et al., 2006). Thus, tackling chronic

inputs of nutrients during low summer flows may provide the greatest gains in terms of improving ecological quality (Shore et al., 2017).

The question remains as to the impact of an acute (diffuse) event, if it also occurs during a period of ecological sensitivity (e.g. summer low flows) when chronic (point) pressures usually dominate. Further, it is projected that due to climate change, storms will increase in frequency and intensity throughout the year, resulting in an increased risk of diffuse pollution (Easterling et al., 2000). It is still unclear what impact this may have on ecosystems which are already under pressure from chronic pollution sources at these times, but it is predicted that increased frequency of extreme events will have serious consequences for ecosystem food webs (Woodward et al., 2016). In addition, little work has been carried out on the impact of chronic versus acute nutrient pressures on ecological communities and this work has focussed on single stressors, most notably phosphorus (e.g. Shore et al., 2017). However, agricultural stressors rarely occur on their own (Ormerod et al., 2010; Townsend et al., 2008), therefore efforts to remediate their effects must reflect a holistic approach which takes into account the impact of multiple stressors acting simultaneously. Thus, there is a need for experimental studies assessing the relative influence of chronic and acute sources of nutrient pollution in a multiple stressor context.

To address the knowledge gaps identified above, a mesocosm experiment was undertaken to investigate the response of stream macroinvertebrate communities to nitrogen, phosphorus and sediment, with the two nutrients being manipulated to simulate the effects of both chronic and acute pollution events, with acute events occurring against a backdrop of chronic pollution. This study builds on a related study (Davis et al., 2018) which examined the effects of the same three stressors by distinguishing between chronic and acute effects of the two

nutrients. Based on findings from this previous study and those of related experiments in New Zealand (Piggott, Townsend, & Matthaei, 2015; Wagenhoff, Townsend, & Matthaei, 2012) and Germany (Elbrecht et al., 2016), it was hypothesised that 1) sediment would be the most pervasive stressor, consistently having a negative effect on sensitive macroinvertebrate taxa in the benthos, 2) sediment addition would lead to an immediate strong drift response but this would diminish as time went on, 3) nutrients would have relatively weak (compared to sediment effects) but predominantly negative effects on sensitive macroinvertebrates in the benthos, 4) nutrients would cause no immediate drift response but such responses would develop with longer exposure, 5) there would be a greater negative effect of acute nutrient enrichment on macroinvertebrates, beyond that seen in channels which only received chronic nutrient enrichment, and 6) the most severe impact would be seen in mesocosms which received sediment coupled with an acute nutrient treatment. To the best of our knowledge, this is the first experiment to investigate the effects of chronic versus acute nutrient enrichment with N and P on stream macroinvertebrate communities.

2. Methods

2.1 Experimental system

The experiment was conducted over 44 days (a 26-day colonisation period followed by an 18-day manipulative period) from 27th April to 9th June 2017 (late spring/early summer) adjacent to the Kildavin River, a first-order stream in county Wexford, south-east Ireland (N 52° 17'24.3", W 06° 31'02.1", 22 m ASL). The Kildavin is representative of small Irish streams mildly impacted by agricultural activities. The upper reaches drain mostly conifer forests, with grassland dominating the areas surrounding the experimental setup. Background nutrient concentrations in the Kildavin (measured twice, once before and once during the experiment) were 0.018 ± 0.006 (standard deviation) mg L^{-1} for dissolved reactive phosphorus (DRP) and 0.904 ± 0.112 mg L^{-1} N for dissolved inorganic nitrogen (DIN). These concentrations were below the Water Framework Directive (WFD) threshold (mean) of 0.025 mg L^{-1} for high P status (Irish Government, 2009) and below the good nitrate status threshold of 1.8 mg L^{-1}, but marginally exceeded the threshold of 0.9 mg L^{-1} for high N status (surrogate mean thresholds defined by the Irish Environmental Protection Agency (EPA) for good and high status (EPA, 2011)).

The experiment was run in 112 circular streamside mesocosms in a setup functionally identical to the *ExStream System* used in previous experiments in Ireland (Davis et al., 2018), New Zealand (Piggott et al., 2015; Wagenhoff et al., 2012) and Germany (Beermann et al., 2018; Elbrecht et al., 2016). Water was continuously pumped from the Kildavin (through a 4-mm mesh filter) via two Pedrollo HF 70 water pumps (Pedrollo, Italy) and split evenly between seven header tanks. Each tank supplied 16 circular mesocosms (external diameter 25 cm, inner outflow diameter 6 cm, volume 3.5 L). Water flow through each channel was maintained

at 2 L min^{-1} (resulting in a complete water exchange within less than 2 minutes) and was calibrated daily. Water exited through a circular opening in the centre of the mesocosm. For an overview of the experimental setup (with 64 channels) see Elbrecht et al. (2016) or Beermann et al. (2018).

At the beginning of the colonisation period, each mesocosm received a standardised amount of substratum, chosen to mimic the streambed of the Kildavin. This substratum was collected from the Kildavin, downstream of the pump intake, dried and sieved before addition. It consisted of 50 mL of sand (0.5-2 mm), 50 mL of gravel (2-4 mm), 300 mL of large gravel (4 mm-30 mm), 4 stones (30-60 mm) and 1 large stone (>60 mm).

Stream biota (macroinvertebrates, algae and microbes) naturally colonised the mesocosms for a period of 26 days. On day 23, natural colonisation was supplemented by adding one standard load of macroinvertebrates in the form of 1/8 equivalent of two two-minute kick samples. This was to ensure that taxa underrepresented in the drift were also present in the mesocosms. Samples for seeding were collected from downstream of the pump intake. Each kick sample was divided between eight channels, by pouring from a 10 L bucket into eight 1 L plastic buckets which were attached to a rotating disc which ensured equal division. Each mesocosm received organisms from an equivalent streambed area of approximately 0.05 m^2 (comparable to the benthic surface area of the mesocosm). Macroinvertebrate density can be assumed to be similar in all mesocosms at the start of the experimental phase (as shown by Magbanua, Townsend, Hageman, and Matthaei (2013) in an experiment run using the same mesocosm setup), or at least not to vary systematically across the randomly assigned stressor treatments. As a result, any effects observed at the end of the manipulative period can be assumed to be the result of the experimental treatments applied.

The manipulative period began on 23rd May 2017. Three stressors were manipulated: sediment (ambient; high), nitrogen (ambient; chronic; acute) and phosphorus (ambient; chronic; acute). Treatments were randomly assigned to each mesocosm in a partial-factorial design with 8 replicates of each treatment combination (see Table 1 and Fig. 1). Sediment was added to allocated treatments on the first day of the manipulative period and chronic nutrient treatments were also applied from this time onwards.

2.2 Nutrient treatments

Mesocosms allocated for chronic nutrient enrichment received a concentrated solution of either nitrate ($NaNO_3$), phosphate (KH_2PO_4) or both. Nutrients were continuously added using a fluid metering pump (BL10, Hanna Instruments, USA) to achieve mean concentrations of 8.04 ± 0.512 mg L^{-1} DIN and 1.01 ± 0.061 mg L^{-1} DRP (measured on day 1, 2, 6 and 13 of the experimental phase, n=128). These concentrations were chosen to mimic high concentrations observed in agricultural streams in Ireland during the summer months (Jordan et al., 2007; Jordan et al., 2012; Melland et al., 2012). Chronic nutrient concentrations were maintained throughout the 18-day manipulative period.

Mesocosms allocated for acute nutrient enrichment received the same concentrated solution of nitrate and/or phosphate as the chronic treatments during the entire manipulative period. Additionally, concentrations of N and/or P were increased in acute channels to 16.11 ± 0.415 mg L^{-1} DIN and 2.04 ± 0.060 mg L^{-1} DRP (measured twice on day 6 and 13 (i.e. during the two acute pulses), n=64) during two three-hour periods on days 6 and 13 of the manipulative phase. Following each pulse, concentrations were returned to chronic enrichment levels.

Acute concentrations were based on concentrations observed in Irish agricultural streams during storms, and the three-hour duration is typical of concentration spikes during moderate to large storms (Jordan et al., 2007; Mellander et al., 2014; Shore et al., 2017). Nutrient concentrations in ambient mesocosms were on average 0.99 ± 0.116 mg L^{-1} DIN and 0.01 ± 0.005 mg L^{-1} DRP (i.e. matching background stream concentrations, measured on day 1, 2, 6 and 13, n=192).

2.3 Fine sediment treatments

Fine sediment was collected from the flood plain of the high-status River Funshion, in the south-west of Ireland. Phosphorus levels in the sediment (analysed using Morgan's P test; Morgan, 1941) were very low at 0.30 – 0.32 ppm (soil P index 1; Wall and Plunkett, 2016). Sediment was oven-dried (12 hours at 80°C) and sieved to a size of 500 µm. There was 240 ml of fine sediment added to mesocosms scheduled to receive sediment, which resulted in sediment cover values equivalent to those which can occur in intensively farmed agricultural streams in Ireland (Conroy et al., 2016). Prior to and during addition, water flow was stopped for approximately 10 minutes to allow the sediment to settle out on the bottom of the mesocosms. Mean sediment cover levels and depths on the final day of sampling (quantified by visual inspection and a ruler, respectively, in mesocosm quarters and using averages per mesocosm) were 5.7 ± 1.5 % with a depth of 0.1 ± 0.4 mm for ambient sediment treatments and 82.3 ± 7.0 % with a depth of 7.7 ± 3.3 mm for high sediment treatments.

2.4 Macroinvertebrate sampling

A nylon net (mesh size 500 µm) was placed over the central opening of each mesocosm to capture any drifting organisms leaving the system on three occasions for 48 hours each: at the start of the manipulative period (days 0-2), immediately prior to the first acute pulse (days 6-7), and prior to the second acute pulse (days 13-14). The first of these sampling periods was chosen as previous studies have shown the first 24-48 hours after addition to be the key time for drift responses to sediment (Conroy et al., 2016; Larsen & Ormerod, 2010; Larsen, Pace, & Ormerod, 2011; O'Callaghan, Jocque, & Kelly-Quinn, 2015; Suren & Jowett, 2001). The second and third period aimed to determine if acute nutrient pulses caused an immediate drift response due to the high concentrations applied. Benthic macroinvertebrates were sampled at the end of the manipulative phase (day 18) by collecting the entire substratum of each mesocosm. All samples were immediately preserved in 70% industrial methylated spirit (IMS) for later analysis. In the laboratory, all samples were sorted and identified to the lowest practicable taxonomic level (species or genus for most Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Mollusca and Crustacea; family for Diptera, and class for Oligochaeta) using standard Freshwater Biological Association identification keys (Dobson, Pawley, Fletcher, & Powell, 2013; Edington & Hildrew, 1995; Elliott & Humpesch, 2010; Hynes, 1977; Wallace, Wallace, & Philipson, 1990).

2.5 Statistical Analysis

Data were analysed using SPSS 24 (IBM SPSS Statistics; IBM Company, Chicago, IL, U.S.A.). The responses of six community-level macroinvertebrate metrics were examined for all benthic and drift data: total macroinvertebrate abundance, total taxon richness, EPT abundance, EPT richness, Simpson's diversity and Pielou's evenness. For each of these, a three-factor ANOVA was carried out, with sediment, phosphorus and nitrogen as fixed factors. The model of this ANOVA was intercept (d.f. 1) + sediment (1) + nitrogen (2) + phosphorus (2) + sediment×nitrogen (2) + sediment×phosphorus (2) + nitrogen×phosphorus (2) + sediment×nitrogen×phosphorus (2) + error (98, n=112). This was a partial factorial design as the levels chronic N and acute P, and chronic P and acute N did not appear in combination with one another. To evaluate the biological relevance of results, standardised effect sizes (partial eta squared values, range 0-1; Garson 2012) were calculated. For all significant stressor main effects and all significant stressor interactions, post-hoc tests were carried out using Tukey's Honest Significant Difference (HSD) to determine pair-wise differences and interaction shapes.

The ten most common taxa in the benthos at the end of the experiment and in each set of drift samples were also analysed. These taxa accounted for 98.0% of all individuals counted in the benthos, 89.4% in the first 48-hour drift, 93.4% following the first acute pulse and 91.1% following the second acute pulse.

To assess stressor effects on macroinvertebrate community composition in the benthos and in the drift, four separate (one benthos and three drift) three-factor MANOVAs were carried out. All response patterns described below were significant ($P < 0.05$) unless otherwise indicated.

Interpretation of the main effects of the experimental factors concerned (or of lower-order interactions) must be performed with care where significant interactions are present. We followed recommendations of Quinn and Keough (2002) to interpret individual stressor main effects (or 2-way interactions in the presence of a 3-way interaction) only where the effect size of the 2-way interaction (or the 3-way interaction) was smaller than the size of the corresponding main effects (or 2-way interactions).

3. Results

A total of 52,307 macroinvertebrates belonging to 30 taxa were counted and identified, of which 44,647 were from the mesocosm substratum and 7,660 from the drift samples. Each mesocosm substratum contained an average of 396.4 ± 100.2 (SD) individuals and 14.9 ± 2.4 taxa per channel. These taxa included 7.8 ± 1.8 EPT taxa per channel. The Chironomidae were the most abundant benthic taxon with 29,596 specimens counted (66.3% of all individuals) and an average of 246.3 ± 89.0 individuals per channel. Each drift sample contained an average of 22.8 ± 14.7 individuals with a mean taxon richness of 5.9 ± 2.9.

3.1 Benthos

Community-level metrics. Total macroinvertebrate abundance, total taxon richness, EPT abundance and EPT richness were all reduced by added fine sediment as a main effect (Table 2). The latter three metrics also showed three-way interactions between all stressors (Fig. 2). At ambient sediment and ambient N, chronic and acute P enrichment both increased total richness, but this effect became negative when sediment was also added. Likewise, the effect of chronic N at ambient P was reversed in the presence of sediment. Added sediment reduced EPT abundance at all nutrient levels, but this reduction was strongest at ambient N with chronic P and at acute N with acute P. Generally, EPT richness responded positively to nutrient enrichment at ambient sediment, but this response became neutral or slightly negative at high sediment.

None of the community-level metrics was affected by N or P as main effects and there were no significant two-way interactions (Table 2). Evenness and diversity were unaffected by all experimental manipulations.

Community composition and stressor effects on common taxa. The MANOVA indicated that benthic macroinvertebrate community composition was affected by sediment and N as main effects, with no significant interactions among stressors (Table 2). To determine which individual taxa were driving these overall responses, the MANOVA between-subjects effects for the ten most common taxa were examined (Table 2). Eight taxa showed significant stressor main effects and/or interactions between two or three stressors. As a main effect, sediment reduced eight taxa (*Leuctra* sp., *Rhithrogena semicolorata*, *Baetis rhodani*, *Serratella ignita*, *Silo pallipes*, Chironomidae, *Gammarus duebeni*, *Elmis aenea*), with *Dicranota* sp. and *Potamopyrgus antipodarum* being the only taxa not significantly affected by sediment. Nitrogen affected only a single taxon (*G. duebeni*) as a main effect, and P affected none.

The amphipod *G. duebeni* was reduced by both chronic and acute N compared to ambient N, but there was no significant difference between chronic and acute N. This species was also affected by a N×P interaction which overrode a smaller sediment main effect. It responded positively to chronic or acute P at ambient N (Fig. 3). The beetle *E. aenea*, which showed a N×P interaction, was rarest in treatments with acute N plus acute P and most abundant in treatments with acute P only. The caddis *S. pallipes* showed a 3-way interaction. This species was most abundant when either chronic P or chronic N were acting singly, while sediment addition caused a mixed response, with an increase in abundance in the absence of enrichment and mostly negative effects in any nutrient enrichment treatment (Fig. 3).

3.2 Drift during the first 48 hours of the manipulative period

In the first 48 hours following implementation of the sediment and chronic nutrient treatments (i.e. four days before the first acute nutrient pulse), sediment was the dominant driver of macroinvertebrate drift responses. This effect was even stronger than on the benthos, with all eight community-level metrics analysed showing higher numbers drifting in the presence of sediment (Table 3). Sediment also affected drift community composition (see multivariate MANOVA results) and the abundances of all ten common drift taxa analysed, with all taxa showing increased drift at high sediment, apart from Simuliidae. Besides these sediment main effects, drift community composition was affected by a three-way interaction, and both community-level metrics and common taxa showed several three- or two-way interactions, which are described below.

Drift EPT abundance showed a N×P interaction and a weaker three-way interaction. In the N×P interaction, drift was lowest in ambient nutrient treatments and higher in all enriched treatments (note that all nutrient addition treatments represented chronic enrichment at this stage of the experiment), with effects of N enrichment alone generally being worse than when combined with P (Table 3, Fig. 4). In the three-way interaction, EPT drift increased slightly when nutrients were added at ambient sediment or when sediment was added but no nutrients and increased strongly when sediment addition was combined with any type of nutrient enrichment (N, P, or both). EPT drift richness also showed a N×P interaction, with richness being lowest in mesocosms without any enrichment.

The mayfly *Baetis sp*. showed a N main effect (overall drift increased at chronic N compared to ambient N) and a slightly weaker sediment×N interaction (Table 3, Fig. 5). At high sediment *Baetis* drifted more in channels enriched with N than at ambient N, while there was no

difference between N treatments at ambient sediment. The beetle *Hydraena* sp. was affected by a three-way interaction which overrode a weaker sediment×P interaction. Added sediment tended to increase *Hydraena* drift when combined with nutrient enrichment, but this effect was somewhat inconsistent across individual nutrient treatment combinations. The mayfly *R. semicolorata* showed a three-way interaction (with a pattern similar to that for EPT abundance) which overrode a weaker N×P interaction. Numbers drifting were lowest at ambient sediment and ambient nutrients, increased slightly when nutrients were added at ambient sediment or when sediment was added but no nutrients, and increased strongly when sediment addition was combined with nutrient enrichment. Simuliidae were affected by a sediment×P interaction which overrode a similar-sized P main effect. Simuliid drift was highest at ambient sediment, ambient P and ambient N, and lower in all other treatment combinations. At ambient sediment, enrichment with P made it less likely that individuals would drift. Likewise, sediment addition decreased drift tendency with a significant difference between ambient and high sediment at ambient P.

3.3 Drift following the first acute nutrient pulse

Sediment remained the most influential stressor driving macroinvertebrate drift patterns during the 48 hours following the first acute nutrient pulse, but its effects started to reduce (Table 4). Sediment affected two community-level metrics, Simpson's diversity and Pielou's evenness, both of which increased with added sediment. Sediment was the only stressor which affected community composition (Table 4, multivariate MANOVA result), with one interpretable sediment main effect (increased drift) on the common taxon Chironomidae. Phosphorus affected two common taxa (the beetles *L. volckmari* and *Hydraena* sp.) as a main

effect, whereas N had no significant main effects. *Limnius volckmari* drift was higher at chronic P than at acute P or ambient P, while *Hydraena* drift was higher at chronic P compared to ambient P. A significant sediment main effect for *G. duebeni* was overridden by a slightly stronger 3-way interaction. *G. duebeni* drift increased most markedly when sediment addition was combined with either acute P or acute N alone or when chronic N and chronic P were combined (Fig. 6).

3.4 Drift following the second acute nutrient pulse

In the 48 hours following the second pulse, N became the dominant stressor for the drift community, displacing sediment. Nitrogen affected four of the six community-level variables as a main effect, whereas sediment affected none (Table 5). Total drift abundance and total richness both increased at chronic N compared to ambient N, while EPT abundance and richness increased at chronic and acute N compared to ambient. Phosphorus affected two community-level variables as a main effect. Simpson's diversity was higher at chronic and acute P compared to ambient, whereas EPT drift abundance was higher at chronic P compared to ambient. There was a N×P interaction for total abundance, with increased drift at chronic N when combined with chronic P compared to ambient nutrients or all other nutrient treatment combinations (Fig. 7).

Drift community composition was affected by both N and P as main effects, but not by sediment (Table 5, multivariate MANOVA results). A single common taxon, *Baetis* sp., did respond to sediment addition, with more individuals drifting at ambient than at high sediment. Three common taxa (Chironomidae, *G. duebeni* and *R. semicolorata*) were affected

by N (Fig. 7). Chironomidae drift was higher at chronic than at acute N while for both *R. semicolorata* and *G. duebeni,* drift was higher at chronic or acute N compared to ambient. Hydrachnidia showed a N×P interaction that overrode a smaller P main effect, with the highest drift in mesocosms with ambient nutrients and lower drift in all other nutrient treatment combinations. *R. semicolorata* was affected by a three-way interaction. The species drifted least in mesocosms with ambient sediment and ambient nutrients, whereas its drift abundances were highest in treatment combinations which received either chronic N plus chronic P or a combination of all three stressors

4. Discussion

4.1 Fine sediment

Our first hypothesis was supported, as sediment addition negatively affected four of six macroinvertebrate community-level metrics and eight of ten common taxa in the mesocosm benthos. Similarly, sediment was the dominant driver of total macroinvertebrate drift in the first 48 hours of the manipulative phase and led to increased drift for all six community-level metrics and for nine of the ten common drift taxa. Sediment continued to be the dominant driver of drift responses following the first acute nutrient enrichment pulse (day 6-7), but effects were much less pronounced than in the first 48 hours. Following the second acute pulse (day 13-14), the effect of sediment on drift responses had almost disappeared, confirming our second hypothesis.

The observed declines in the pollution-sensitive EPT taxa in the first 48 hours in response to sediment addition agree with previous streamside channel experiments which found that

negative effects of fine sediment tended to lead to increased drift immediately following addition (Davis et al., 2018; Magbanua, Townsend, Hageman, Piggott, & Matthaei, 2016; O'Callaghan et al., 2015; Suren & Jowett, 2001). EPT taxa are particularly sensitive to in-stream sedimentation as many species have exposed and delicate gill structures which can be easily damaged or clogged by sediment (Jones et al., 2012). Sediment can also smother habitats (Soulsby et al., 2001; Wood & Armitage, 1997), reduce available food for grazers (Wood & Armitage, 1997), and lead to oxygen depletion (Jones et al., 2012; Wood & Armitage, 1997).

Our finding that most of the drift responses to added fine sediment occurred in the first 48 hours is important for freshwater management, as this demonstrates that sediment deposition can potentially have major detrimental effects on local stream macroinvertebrate communities in a very short period of time. Equally, the strong response to sediment found in the benthos at the end of the 18-day manipulative period shows that the macroinvertebrate community did not recover while sediment was still present on the mesocosm bed, despite most of the drift response disappearing within seven days. This result agrees with other mesocosm studies that found sediment was a strong driver of macroinvertebrate drift responses which were reflected by decreased numbers in the benthos (Davis et al., 2018; Magbanua et al., 2016; Piggott et al., 2015).

4.2 Nutrients

Our third hypothesis was supported by the results in the benthos, as P had no significant main effects and only one taxon showed an interpretable main effect for N (negative effect on *G.*

duebeni). However, nutrient enrichment effects were more common in the drift and became more pronounced the longer the community was exposed, supporting our fourth hypothesis. Only one macroinvertebrate drift variable responded to N as a main effect in the first 48-hour drift, and there was no drift response to N following the first acute pulse. Following the second acute pulse, by contrast, eight of 17 drift variables were significantly affected by N, making N the dominant driver of drift responses two weeks after stressor implementation. The effects of P also increased as the experiment went on, with no interpretable main effects in the first 48-hour drift, two following the first acute pulse, and four following the second acute pulse. Interestingly, these increasing drift responses over time were not reflected in the benthos as hardly any significant main effects of N or P were observed (see above). Had the manipulative period been longer than 18 days, the negative effects of chronic N and P enrichment which appeared in the drift after two weeks might have also manifested themselves in the benthos. Longer multiple-stressor mesocosm experiments (ideally over several months) would allow one to more comprehensively assess the effects of nutrient enrichment on stream macroinvertebrate communities, even though one of the first such experiments, conducted by Graeber et al. (2017) in Denmark, found no significant response of macroinvertebrate community composition to eight weeks of nutrient enrichment.

Our fifth hypothesis was rejected by our findings as only two responses showed a significant difference between chronic and acute nutrient levels and in both cases the effects of chronic enrichment were worse than acute. Of the remaining 12 interpretable nutrient main effects, seven showed differences between ambient compared to both chronic and acute levels, but no difference between chronic and acute, while five showed differences between chronic and ambient only. These results suggest that chronic enrichment caused most of the observed

negative effects and that acute nutrient pulses caused no significant effects above and beyond those due to chronic enrichment. Nitrogen enrichment levels in our experiment greatly exceeded the threshold of 1.8 mg L^{-1} N for good status in Ireland (EPA, 2011), but the relatively short manipulative period (18 days) was probably not sufficient to detect the many indirect consequences of eutrophication (Camargo & Alonso, 2006) or changes in competitive interactions among macroinvertebrates because of alterations among food availability (Evans-White, Dodds, Huggins, & Baker, 2009). Therefore, the increasingly negative effects of N on macroinvertebrate drift seen towards the end of our experiment were likely due to chronic toxicity rather than eutrophication symptoms, suggesting that the length of exposure to elevated nutrient concentrations may be more important than the actual concentrations. This theory is supported by the fact that only one significant difference was detected between chronic and acute N treatments, suggesting that in the short-term even doubling concentrations beyond the already high chronic levels did not have a measurable additional impact.

These findings have important implications for management which often focusses on the concentrations of nutrients at a particular point in time (i.e. one spot sample) rather than the duration of exposure to elevated concentrations. They also suggest managing chronic sources of nutrient pollution, which are usually associated with point sources (Bowes et al., 2008), may provide the greatest gains in terms of improving ecological quality compared to acute sources. Although the nutrient concentrations in our study were relatively high, they reflect levels which can typically be seen during summer low-flows (chronic) and large precipitation events (acute) in Irish agricultural catchments (Jordan et al., 2007; Jordan et al., 2012; Melland et al., 2012; Mellander et al., 2014; Shore et al., 2017). During summer, these low-flow related

increases in nutrient concentrations can last for several months. Additionally, the effects of climate change are predicted to lead to increases in the frequency of droughts as well as increases in frequency and magnitude of storms (Easterling et al., 2000; Trenberth, 2011).

4.3 Complex multiple-stressor responses

Interactions among the manipulated stressors affected 27% (17 of 64) of all studied macroinvertebrate response variables. Of these, the most complex were the three-way interactions between sediment and the two nutrients, which affected 14% of all response variables overall. These interactions were most frequent in the benthos where they affected 25% of response variables, compared with 19% in the first 48-hour drift and 6% following each of the two acute nutrient pulses. While the shapes of these interactions varied, in general nutrient enrichment exacerbated the negative effect of sediment addition, resulting in stronger responses in the benthos and the three sets of drift data when one or both nutrients were added in combination with sediment. These findings are similar to previous reach-scale or streamside channel experiments which often found that nutrient enrichment worsened the negative effects of added sediment (Matthaei et al., 2010; Townsend et al., 2008; Wagenhoff et al., 2012).

Our sixth hypothesis was rejected for both two- and three-way interactions, paralleling the findings for our fifth hypothesis which had been rejected as well, probably due to similar reasons (as discussed above). Although several complex interactions occurred between sediment and nutrients in the benthos and some of these were synergistic, there was no clear evidence suggesting that acute treatments had a greater effect than chronic treatments when

combined with sediment. In the drift, nutrients and sediment generally interacted synergistically, but again there were no clear differences between chronic and acute nutrient levels in combination with sediment.

4.4 Conclusions and management implications

To our knowledge, this is the first experiment to investigate the effects of chronic versus acute nutrient enrichment with N and P on stream macroinvertebrate communities. Despite some limitations in the realism of the experimental setup (discussed in detail in Elbrecht et al., 2016 and Beermann et al., 2018), our study provides novel insights into how stream macroinvertebrates respond to multiple stressors. Our findings imply that, while management of both sediment and nutrient inputs is key to achieving and maintaining streams in good ecological status, priority should be given to managing inputs of sediment. Deposited fine sediment was the most pervasive stressor in our study, confirming a growing number of studies in Ireland (Bruen et al., 2017; Conroy et al., 2016; Davis et al., 2018; Lawler et al., 2016) and elsewhere (e.g. Beermann et al., 2018; Elbrecht et al., 2016; Matthaei et al., 2010; Wagenhoff et al., 2012), suggesting that sediment is the most serious threat to the ecological integrity of agricultural streams.

We detected only two significant differences between chronic and acute nutrient treatments for N or P. Negative effects of either nutrient generally appeared to be a result of chronic exposure, with acute pulses having no additional adverse effect. Therefore, management of nutrients pressures should initially focus on chronic inputs, which tend to originate from point sources and affect rivers over longer periods. These inputs tend to be particularly prevalent

during summer when water levels are low and nutrient concentrations increase. Additionally, sediment tends to persist on the streambed during summer baseflows. Consequently, cost-effective mitigation measures should be targeted to these critical times of year, but priority should be given to addressing sediment pressures before focussing on nutrients. Further, the duration of exposure to nutrients and deposited sediment should be considered, something often overlooked by typical spot-sample monitoring programmes.

In terms of future research, studies on the effects of acute nutrient pulses against a backdrop of ambient nutrient concentrations are needed. This scenario would more accurately simulate the effects of winter/spring storms than our experiment. Further multiple-stressor studies manipulating chronic nutrient pressures for several months are also required, as discussed earlier.

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Table 1. The 14 treatment combinations of the three stressors manipulated in the experiment. There were eight replicates of each treatment combination. Note that the treatment levels chronic N and acute P, and chronic P and acute N did not appear in combination with one another in this partial factorial design.

Sediment	Nitrogen	Phosphorus	Replicates
Ambient	Ambient	Ambient	8
Ambient	Acute	Ambient	8
Ambient	Chronic	Ambient	8
Ambient	Ambient	Acute	8
Ambient	Acute	Acute	8
Ambient	Ambient	Chronic	8
Ambient	Chronic	Chronic	8
High	Ambient	Ambient	8
High	Acute	Ambient	8
High	Chronic	Ambient	8
High	Ambient	Acute	8
High	Acute	Acute	8
High	Ambient	Chronic	8
High	Chronic	Chronic	8

Table 2. Macroinvertebrate responses in the mesocosm benthos to the experimental manipulations (summary of (M)ANOVA results, *P*-values presented, with effect sizes (partial eta^2, range 0-1) in brackets for results where $P < 0.1$). Significant *P*-values ($P < 0.05$) are printed in bold. For stressor main effects, arrows indicate an increase or decrease under the stressor condition compared to ambient. Post-hoc test rankings (lowest to highest mean) for Tukey's HSD tests are given in brackets after effect sizes for significant nutrient main effects (0 = ambient, C = chronic, A = acute). ☒ Stressor main effect (or 2-way interaction) overridden by similar-sized or stronger 2-way or 3-way interaction.

	%	S	N	P	S×N	S×P	N×P	S×N×P
Total abundance	100	**<0.001** (0.27) ↓	0.126	0.749	0.980	0.550	0.966	0.468
Total richness		**<0.001** (0.10) ↓	0.252	0.144	0.892	0.340	0.880	**0.040** (0.06)
EPT abundance	8.2	**<0.001** (0.36) ↓	0.224	0.706	0.602	0.395	0.752	**0.019** (0.08)
EPT richness		**<0.001** (0.18) ↓	0.485	0.281	0.603	0.296	0.892	**0.026** (0.07)
Simpson's Diversity		0.153	0.442	0.663	0.949	0.619	0.091 (0.048)	0.954
Evenness		0.140	0.327	0.700	0.956	0.838	0.144	0.951
Community Composition (MANOVA, 10 taxa)	98.1	**<0.001** (0.57)	**<0.001** (0.22)	0.710	0.353	0.750	0.056 (0.15)	0.138
B. rhodani	0.7	**<0.001** (0.18) ↓	0.051 (0.06)	0.750	0.324	0.936	0.432	0.925
Chironomidae	66.3	**<0.001** (0.10) ↓	0.176	0.965	0.991	0.973	0.240	0.875
Dicranota sp.	0.9	0.054 (0.04) ↓	0.171	0.884	0.852	0.166	0.538	0.956
E. aenea	1.4	**<0.001** (0.24) ↓	0.401	0.925	0.457	0.291	**0.043** (0.06)	0.168
G. duebeni	11.2	**0.008** (0.07) ↓ ☒	**0.003** (0.11; (CN=AN) < 0N)	0.067 (0.05)	0.273	0.272	**0.005** (0.10)	0.663
Leuctra sp.	0.8	**<0.001** (0.18) ↓	0.184	0.614	0.980	0.779	0.274	0.369
P. antipodarum	11.5	0.109	0.219	0.442	0.731	0.330	0.299	0.877
R. semicolorata	2.2	**<0.001** (0.12) ↓	0.097 (0.05)	0.760	0.062 (0.06)	0.594	0.530	0.186
S. ignita	0.9	**<0.001** (0.14) ↓	0.986	0.212	0.203	0.536	0.456	0.144
S. pallipes	2.3	**<0.001** (0.10) ↓	0.880	0.590	0.282	0.214	0.101 (0.05)	**0.019** (0.08)

Table 3. Macroinvertebrate drift responses during the first 48 hours following implementation of the sediment and chronic nutrient treatments (*P*-values of (M)ANOVA results, with effect sizes in brackets for results where $P < 0.10$). For further details see legend of Table 1.

	%	S	N	P	S×N	S×P	N×P	S×N×P
Total abundance	100	**<0.001** (0.43) ↑	0.569	0.907	0.131	0.791	0.415	0.375
Total richness		**<0.001** (0.55) ↑	0.276	0.260	0.126	0.552	0.124	0.094 (0.05)
EPT abundance	14.5	**<0.001** (0.60) ↑	**0.018** (0.08; 0N < CN) ☒	0.423	**0.050** (0.06) ☒	0.718	**<0.001** (0.14)	**0.011** (0.09)
EPT richness		**<0.001** (0.57) ↑	0.069 (0.05)	0.476	0.341	0.985	**0.003** (0.11)	0.101
Simpson's diversity		**<0.001** (0.22) ↑	0.396	0.515	0.933	0.437	0.588	0.395
Evenness		**0.032** (0.05) ↑	0.636	0.760	0.919	0.601	0.685	0.620
Community Composition (MANOVA, 10 taxa)		**<0.001** (0.70)	0.203	0.137	0.360	0.212	0.282	**0.008** (0.18)
Baetis sp.	3.2	**<0.001** (0.22) ↑	**0.004** (0.11; 0N < CN)	0.782	**0.031** (0.07)	0.929	0.337	0.610
Chironomidae	36.3	**0.006** (0.07) ↑	0.846	0.723	0.282	0.852	0.496	0.321
E. aenea	9.6	**<0.001** (0.45) ↑	0.487	0.954	0.686	0.934	0.363	0.767
G. duebeni	12.0	**<0.001** (0.44) ↑	0.371	0.692	0.543	0.512	0.815	0.890
Hydrachnidia	9.0	**0.002** (0.09) ↑	0.740	0.737	0.338	0.945	0.317	0.207
Hydraena sp.	3.2	**<0.001** (0.14) ↑	0.315	0.099 (0.05)	0.653	**0.023** (0.07) ☒	0.292	**<0.001** (0.13)
L. volckmari	2.3	**<0.001** (0.14) ↑	0.052 (0.06)	0.245	0.398	0.534	0.376	0.111
R. semicolorata	5.2	**<0.001** (0.53) ↑	0.321	0.192	0.513	0.401	**0.005** (0.10) ☒	**0.007** (0.10)
S. ignita	1.3	**<0.001** (0.18) ↑	0.752	0.143	0.746	0.272	0.766	0.491
Simuliidae	11.1	**<0.001** (0.12) ↓	0.177	**0.023** (0.07; CP < 0P) ☒	0.080 (0.05)	**0.025** (0.07)	0.272	0.222

Table 4. Macroinvertebrate drift responses in the 48 hours following the first acute nutrient enrichment pulse (P-values of (M)ANOVA results, with effect sizes in brackets for results where P < 0.10). For further details see legend of Table 1.

	%	S	N	P	S×N	S×P	N×P	S×N×P
Total abundance	100	0.190	0.410	0.745	0.734	0.466	0.870	0.929
Total richness		0.421	0.382	0.605	0.671	0.186	0.200	0.249
EPT abundance	5.5	0.158	0.407	0.517	0.750	0.505	0.243	0.242
EPT richness		0.069 (0.03)	0.370	0.546	0.731	0.747	0.101	0.274
Simpson's diversity		**<0.001** (0.10) ↑	0.927	0.501	0.632	0.892	0.998	0.261
Evenness		**<0.001** (0.13) ↑	0.782	0.537	0.527	0.930	0.873	0.419
Community Composition (MANOVA, 10 taxa)		**0.042** (0.18)	0.356	0.409	0.999	0.784	0.722	0.587
Baetis sp.	2.4	0.909	0.402	0.772	0.784	0.051 (0.06)	0.187	0.945
Chironomidae	65.0	**0.015** (0.06) ↓	0.526	0.854	0.513	0.611	0.986	0.844
E. aenea	4.6	0.912	0.255	0.375	0.919	0.465	0.812	0.471
G. duebeni	5.5	**0.005** (0.08) ↑ ☒	0.424	0.755	0.999	0.977	0.166	**0.013** (0.09)
Hydrachnidia	10.1	0.159	0.626	0.598	0.583	0.775	0.523	0.740
Hydraena sp.	0.7	0.310	0.127	**0.029** (0.07; (0P=AP) < CP)	0.791	0.941	0.194	0.333
L. volckmari	0.6	0.274	0.656	**0.020** (0.08; 0P < CP)	0.254	0.834	0.087 (0.05)	0.725
R. semicolorata	0.6	0.923	0.246	0.626	0.677	0.573	0.914	0.266
S. ignita	0.5	0.679	0.474	0.697	0.974	0.662	0.662	0.539
Simuliidae	6.0	0.893	0.150	0.586	0.893	0.877	1.000	0.578

Table 5. Macroinvertebrate drift responses in the 48 hours following the second acute nutrient enrichment pulse (*P*-values of (M)ANOVA results, with effect sizes in brackets for results where $P < 0.10$). For further details see legend of Table 1.

	%	S	N	P	S×N	S×P	N×P	S×N×P
Total abundance	100	0.184	**0.018** (0.08; 0N < CN)	0.998	0.999	0.836	**0.048** (0.06)	0.115
Total richness		0.344	**0.014** (0.08; 0N < CN)	0.388	0.849	0.872	0.482	0.203
EPT abundance	9.2	0.130	**<0.001** (0.15; 0N < (CN=AN))	**0.029** (0.07; 0P < CP)	0.184	0.270	0.650	0.480
EPT richness		0.513	**<0.001** (0.16; 0N < (CN=AN))	0.069 (0.05)	0.555	0.821	0.839	0.133
Simpson's diversity		0.711	0.194	**0.029** (0.07; 0P < (AP=CP))	0.852	0.911	0.707	0.942
Evenness		0.758	0.079 (0.05)	0.152	0.770	0.793	0.626	0.515
Community Composition (MANOVA, 10 taxa)		0.280	**<0.001** (0.21)	**0.031** (0.16)	0.335	0.285	0.094 (0.14)	0.464
Baetis sp.	4.3	**0.014** (0.06) ↓	0.094 (0.05)	0.157	0.064 (0.05)	0.089 (0.05)	0.411	0.783
Chironomidae	56.0	0.115	**0.018** (0.08; AN < CN)	0.330	0.959	0.685	0.062 (0.06)	0.363
E. aenea	5.7	0.454	0.570	0.331	0.693	0.609	0.994	0.751
G. duebeni	17.9	0.839	**<0.001** (0.14; 0N < (AN=CN))	0.469	0.977	0.605	0.637	0.239
Hydrachnidia	1.5	0.298	0.158	**0.004** (0.10; CP < 0P) ☒	0.152	0.058 (0.06)	**0.004** (0.11)	0.422
Hydraena sp.	0.6	0.103	0.230	0.393	0.408	0.703	0.590	0.331
L. volckmari	0.4	0.344	0.272	0.687	0.461	0.687	0.321	0.931
R. semicolorata	1.3	0.430	**<0.001** (0.14; 0N < (CN=AN))	**0.006** (0.10; 0P < (AP=CP))	0.297	0.198	0.086 (0.05)	**0.018** (0.08)
S. ignita	1.3	0.639	0.169	0.343	0.375	0.406	0.525	0.443
Simuliidae	7.3	0.717	0.235	0.383	0.673	0.307	0.888	0.593

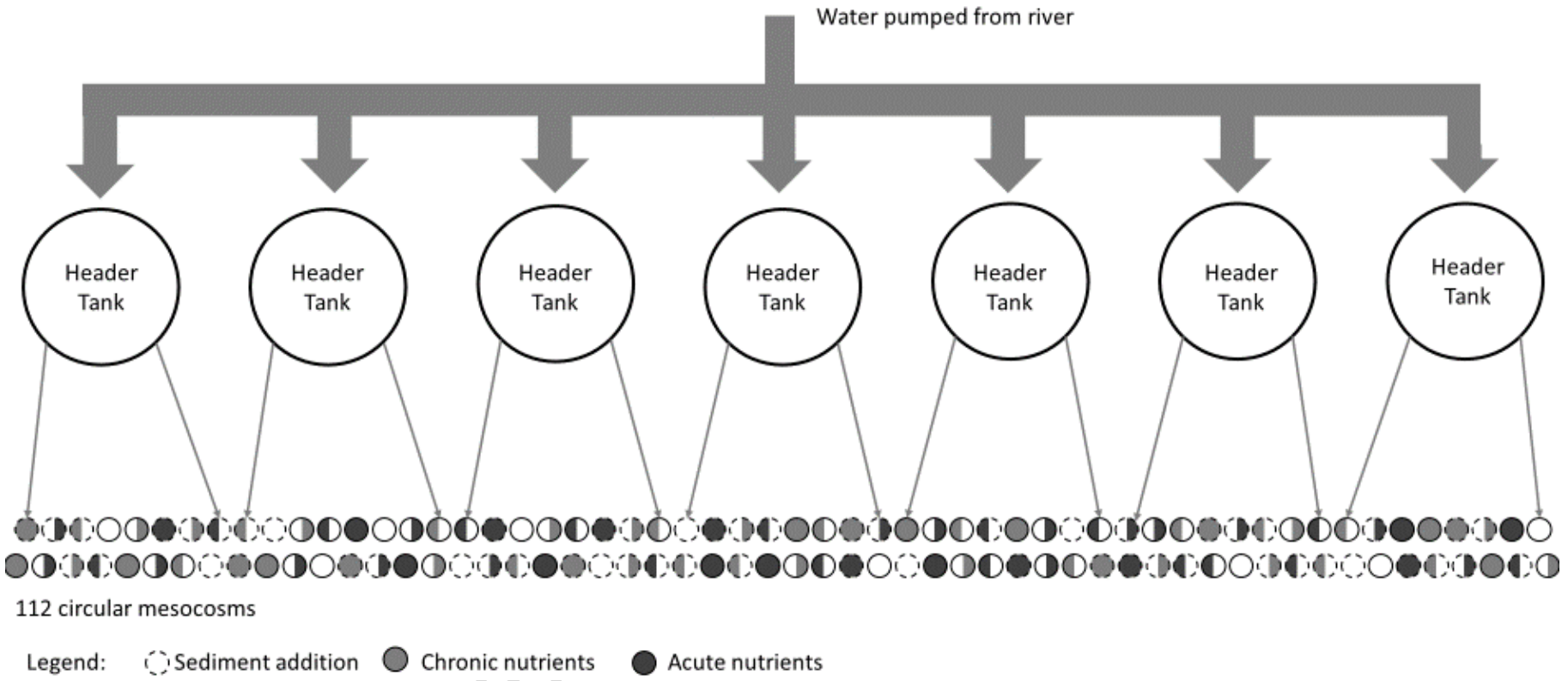

Figure 1. Schematic overview of the experimental mesocosm setup. Stressor combinations in mesocosms are visualised by dashed circles (sediment addition), light grey colouring (chronic nutrient addition) and dark grey colouring (acute nutrient addition). White colouring indicates ambient nutrient concentrations. The left side of each circle represents nitrogen levels and the right side represents phosphorus levels.

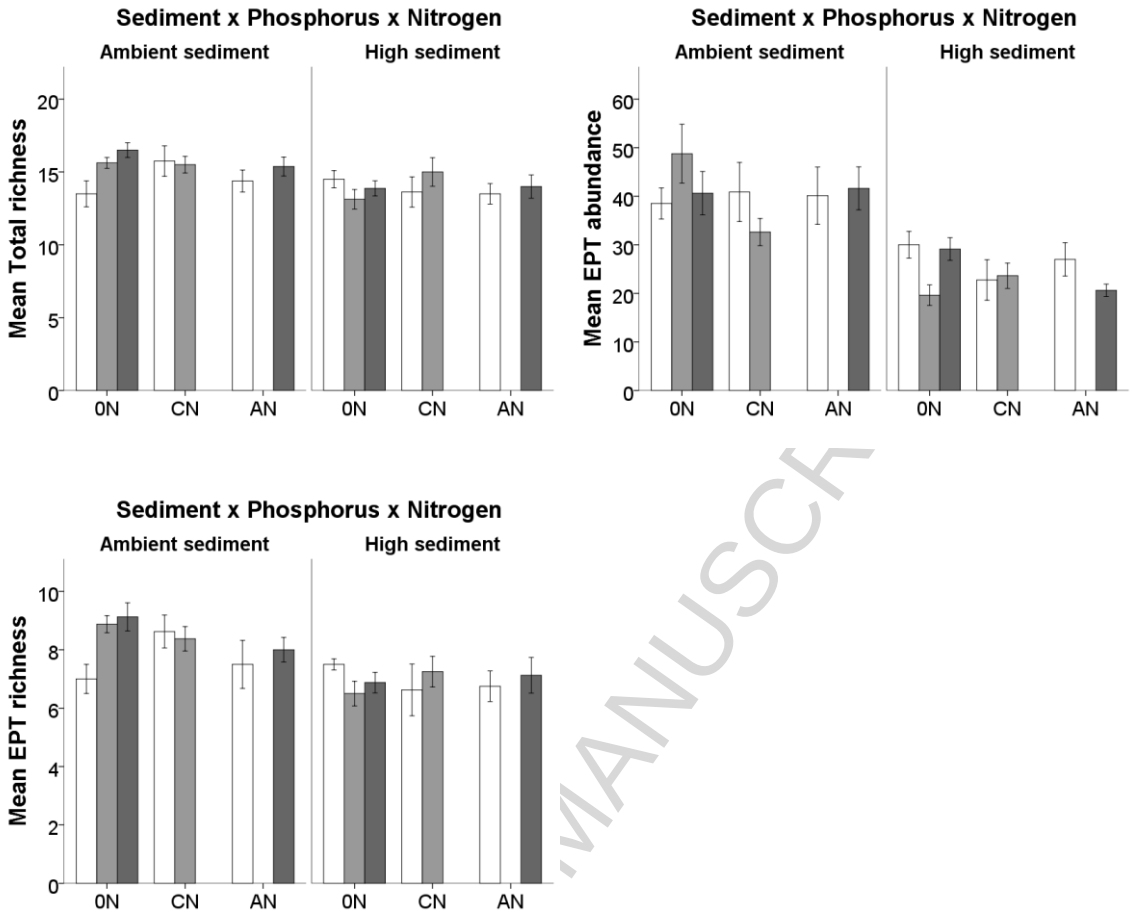

Figure 2. Responses of total taxon richness, EPT abundance and EPT richness in the benthos to the stressor treatments. White bars represent treatments with ambient P, light-grey bars represent treatments with chronic P and dark-grey bars represent treatments with acute P enrichment. Nitrogen treatments are ambient (0N), chronic (CN) and acute (AN). The treatment combinations chronic N or P and acute N or P are missing due to the partial-factorial nature of the experimental design (see Methods). Error bars represent standard errors. Sample size for each treatment combination is n = 8.

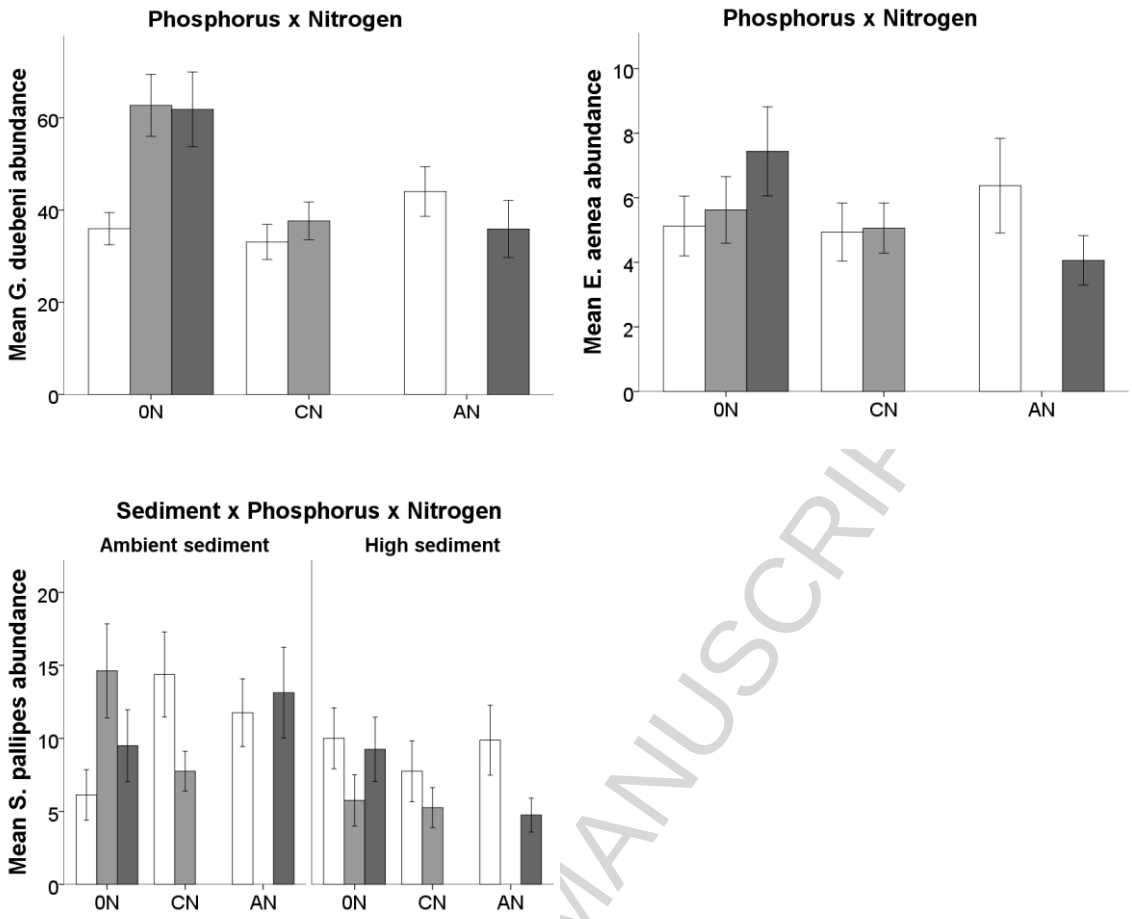

Figure 3. Responses of *G. duebeni, E. aenea* and *S. pallipes* in the benthos to the stressor treatments. Sample size for each P × N interaction is n= 16. Sample size for the three-way interaction is n = 8. For further details see legend of Figure 2.

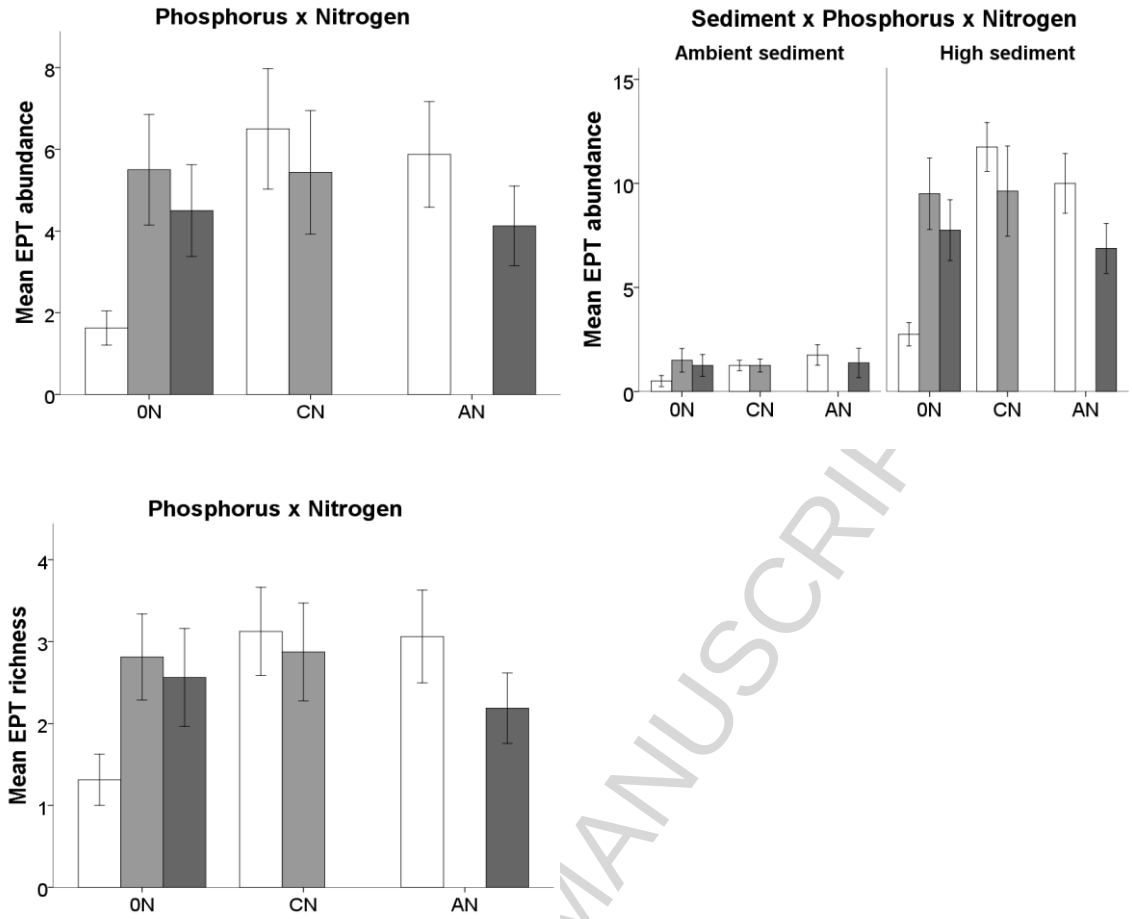

Figure 4. Drift responses of EPT abundance and EPT richness during the first 48 hours after implementation of the sediment and chronic nutrient treatments. Sample size for P × N interaction is n = 16. Sample size for the three-way interaction is n = 8. For further details see legend of Figure 1.

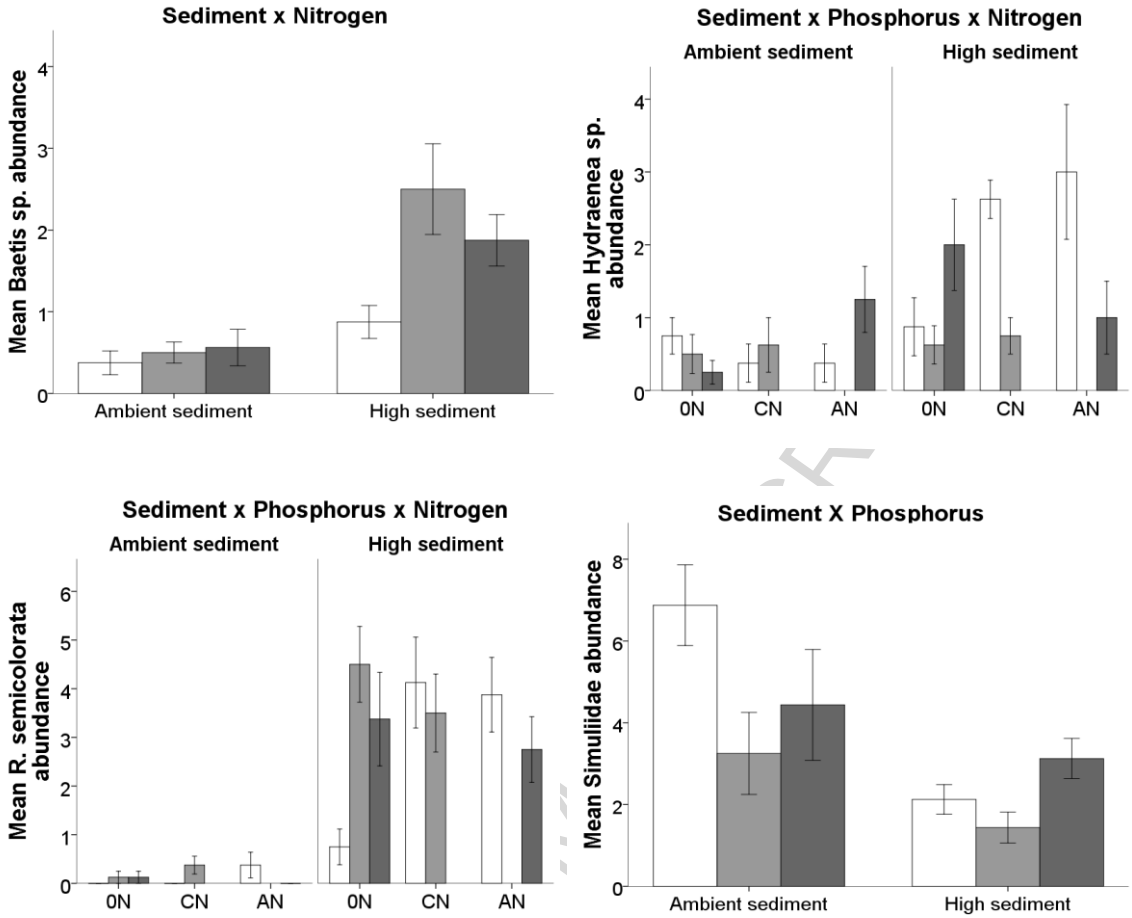

Figure 5. Drift responses of *Baetis* sp., *Hydraena* sp., *R. semicolorata* and Simuliidae during the first 48 hours after implementation of the sediment and chronic nutrient treatments. For each 2-way interaction sample size is 0 = 24, C = 16 and A = 16. Sample size for each three-way interaction is n = 8. For further details see legend of Figure 1.

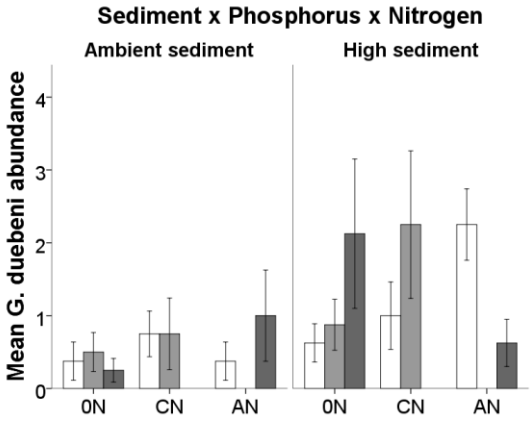

Figure 6. Drift responses of *G. duebeni* following the first acute nutrient pulse. Sample size for each treatment combination is n = 8. For further details see legend of Figure 1.

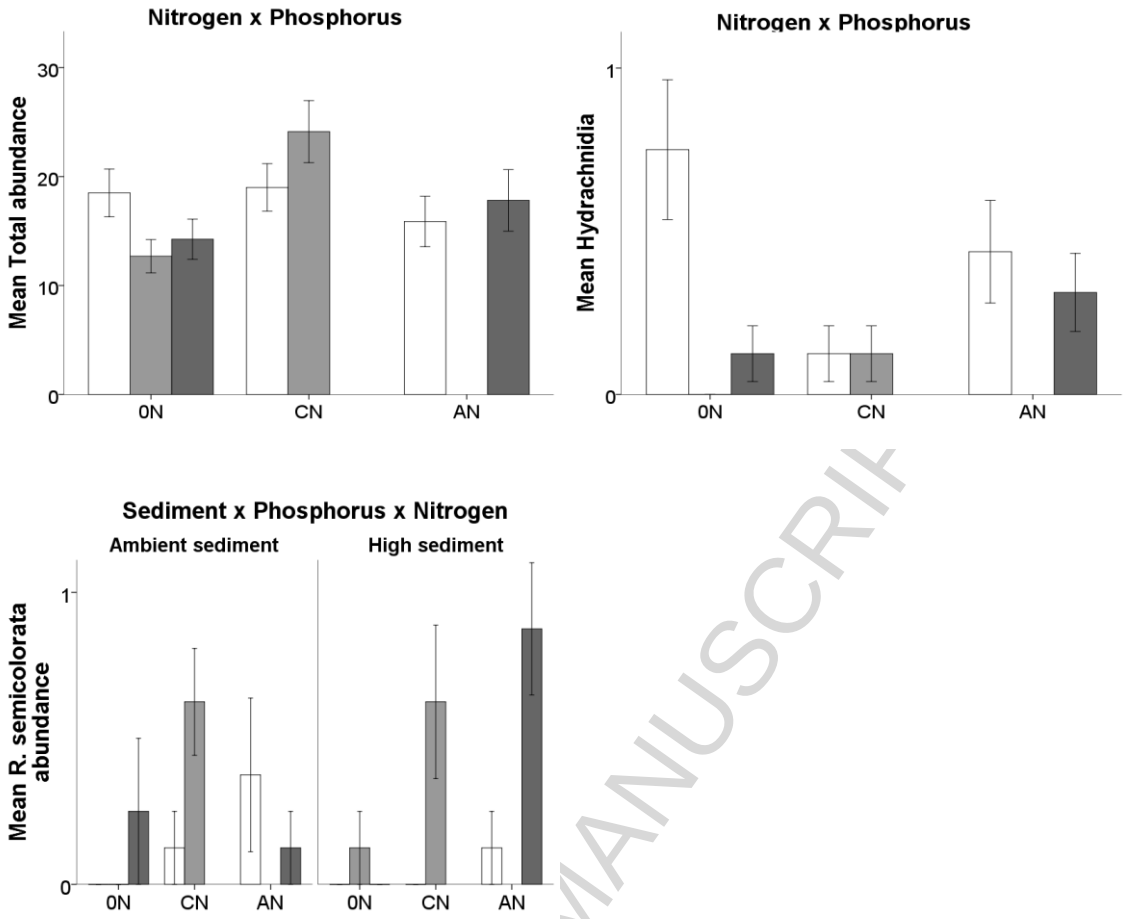

Figure 7. Drift responses of total drift abundance, Hydrachnidia and *R. semicolorata* following the second acute nutrient pulse. Sample size for N × P interaction is N = 16. Sample size for each three-way interaction is n = 8. For further details see legend of Figure 1.